In "Mycologia Memoir No. 11, Indigenous Fermented Food of Non-Western Origin", eds. C. W. Hesseltine and Hwa L. Wang

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Chapter 16

Nutritional Quality of Fermented Foods

by

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A great number of fermented foods primarily derived from plant materials have been an indispensable dietary element in many parts of the world. In the Far East where fermented foods have a long and strong tradition, these foods are uniquely made from soybeans and molds. The fermentation, however, may have a substrate consisting of both soybeans and cereals and an inoculum consisting of yeasts, bacteria, and molds. In other parts of the world, bacteria and yeasts traditionally dominate the fermentation of locally grown cereal grains or cereal grains supplemented with a locally grown legume or milk product. At a relatively low cost, these traditional processes have converted plant materials into culturally acceptable foods that are palatable, safe, economical, and often nutritious.

Many traditional fermented foods are the result of natural fermentations. Natural selection probably has eliminated the microorganisms that were toxic or had other undesirable characteristics. The process could reduce or remove undesirable factors and unpleasant odor and flavor either by soaking and cooking in preparing substrate for fermentation (Wang and Hesseltine, 1981) or by enzymatic hydrolysis during fermentation (Sudarmadji and Markakis, 1977; Wang et al., 1980). In many cases, it also reduces the cooking time. The fermentation has often achieved the purpose of preservation because a number of foods are made with the addition of salt, which acts as a preservative and also has a selective action on the microorganisms. In fermentation by lactic acid bacteria or acid-producing species of Mucorales, organic acids are produced that act as preservative either through lowering the pH or as bacteriostatic agent which inhibits the growth of bacteria. Molds such as *Rhizopus*, *Actinomucor*, and *Monascus* furthermore, were found to produce antibiotics that are active against many-gram-positive bacteria (Wang et al., 1969, 1972).

Consumers consider these fermented foods nourishing and easily digestible, but the question for some time has been whether or not fermentation improves nutritional value. This chapter reviews the present knowledge on the nutritive value of fermented foods.

Effect of Fermentation on Compositional Changes of Substrates

The only traditional fermented food that has been extensively studied in the West is tempeh, which originated in Indonesia and is made from dehulled and briefly cooked soybeans fermented with the mold *Rhizopus*. These studies (Steinkraus et al., **1960**; Wang and Hesseltine, **1966**; Wang et al., **1968**) revealed that fermentation generally increases soluble solids, soluble nitrogen, and free amino acids; whereas total nitrogen remaining fairly constant. The amino acid values of tempeh, fermented up to 30 hours, do not show any significant changes (Smith et al., **1964**; Stillings and Hackler, **1965**) from that of defatted meal and cooked cotyledons from the same beans within the errors of amino acid assays. Perhaps, the amount of microbial protein present in the fermented products was not high enough to change the amino acid composition of the substrate protein. Steinkraus et al. (**1961**), however, have found losses of methionine of about 4 and 11% in tempeh after 36 and 60 hours of fermentation, respectively, and losses of lysine of 11 and 24% for the same period.

The fermentation may slightly alter the proximate composition (%) of the substrate as exemplified in Table I (Wang et al., **1968**). A slight increase in the percentage of protein can be noted. This increase reflects the decrease of other constituents which the mold might have consumed for growth. The decrease of carbohydrates in wheat after fermentation suggested that the mold had used the carbohydrates as an energy source. Sorenson and Hesseltine (**1966**) reported, however, that *R. oligosporus* (Saito) is unable to use the carbohydrates in soybeans. Instead, the mold uses the soybean oil (ether extract) as its energy source; therefore, a decrease in ether extractable substances is noted. Wagenknecht et al. (**1961**) reported that the mold possesses strong lipase activity and about one-third of soybean oil was hydrolyzed into free fatty acids after a 3-day fermentation. There was no preferential utilization of any fatty acid by the mold except for the depletion of some 40% of the linolenic acid in the later stages of the fermentation.

The effect of tempeh fermentation on other legumes other than soybeans such as chick peas, velvet beans, winged beans, broad beans, and jack beans has been studied by Robinson and Kao (**1978**), and Gandjar and Slamet (**1979**, a, b). Their results also showed that amino acid composition remained virtually unchanged after fermentation.

Table I. Effect of fermentation on the proximate composition of wheat and soybeans.

Grain	Ash	Ether extract	Protein	Fiber	Carbo-hydrates
			%		
Wheat, control	1.7	1.9	17.4	2.6	76.5
Wheat, fermented[a]	1.8	2.0	18.2	3.1	74.9
Soybeans, control	3.4	26.8	47.8	3.9	18.1
Soybeans, fermented	3.3	24.7	48.1	3.1	20.9
Wheat + soy-bean (1:1) control	2.5	12.5	31.6	2.8	50.7
Wheat + soy-bean (1:1) fermented	2.6	12.1	33.1	2.7	49.6

[a]Fermentation time with *Rhizopus oligosporus:* 24 hours.

Data on lactic acid bacteria fermentation showed more variation because many of these studies were carried out by natural fermentation. A significant finding was the increase of methionine content in idli, a soft, spongy, and sour-tasting product from India made by fermenting a mixture of black gram (*Phaseolus mungo* L.) and rice with lactic acid bacteria. Rao (1961) observed a 20% increase in methionine. More recently, Padhye and Salunkhe (1978) found a 60% increase in methionine content after a 20 hours fermentation and a 420% increase after a 45 hours fermentation. On the other hand, Steinkraus et al. (1967) found a decrease in methionine content in idli prepared from mixtures of various proportions of black gram to rice, but a slight increase in both methionine and cystine content was noted in idli prepared from 1:1 proportion of black gram to rice. No appreciable increase in methionine was noted by van Veen et al. (1967). Obviously, more research with pure culture is needed to substantiate the synthesis of methionine in idli fermentation, which could be the most valuable contribution of traditional food fermentation because these foods are usually made from plant materials that are low in sulfur-containing amino acids.

Unlike tempeh fermentation, in which soluble solids and soluble nitrogen increase considerably, in idli fermentation a slight increase in soluble solids was noted, but soluble nitrogen was decreased (Steinkraus et al., 1967).

Protein Quality and Digestibility

The effect of fermentation on protein efficiency ratio (PER) and digestibility is summarized in Table II. Although the enzymes produced by the microorganisms used in fermentation have acted upon the substrate and partly hydrolyzed its constituents into small molecules, the digestibility coefficient of these foods tested by rat assay methods is not significantly different from that of unfermented substrate. As indicated in the previous section, fermentation process does not significantly change the total nitrogen content and the amino acid composition, it is, therefore, not surprising to see that the PER values of fermented foods as determined by rat assay are not significantly different from that of unfermented but properly heat-treated substrate. The increase in PER value of idli observed by Rajalakshmi and Vanaja (1967) may not actually reflect the improvement of protein quality by fermentation because the diets used by these authors for PER determination contained no vitamin supplement other than vitamins A and D. The improved nutritional value of idli, therefore, could be due partly to the vitamin synthesized by the microorganisms during the fermentation.

Wheat is not a traditional substrate for making tempeh in Indonesia, but excellent products can be made from many cereals or their combinations with soybeans (Hesseltine et al., 1967). A significant increase in PER value was observed when wheat or wheat-soybean mixture was fermented by *R. oligosporus* (Table II). The PER value of the mixture is higher than either wheat or soybeans alone. The improved PER value of wheat by fermentation cannot be explained by the amino acid composition, since it was not significantly changed as discussed before, but it may be partly attributed to the increase in the availability of lysine in wheat by fermentation as shown in Table III (Wang et al., 1968). Data obtained from pepsin and pancreatin digestion indicated that the total essential amino acids released from wheat by enzymatic digestion increased about 10% after 24 hours of fermentation. Among the essential amino acids, lysine (15%), and histidine (30%) increased proportionally more than the other amino acids whereas other amino acids remained the same. Possibly, the proteolytic enzyme systems produced by the mold attacked the protein in such a way that more lysine and histine could be made available by the digestive enzymes of

Table II. PER[a] and digestibility before and after fermentation.

	PER		Digestibility coefficient		
Food	Before	After	Before	After	Reference
			%		
Tempeh:					
Soybean	2.63	2.56	86.9	86.2	Hackler et al., 1964
Soybean	2.63	2.64	--	--	Smith et al., 1964
Soybean	2.17	2.27	--	--	Wang et al., 1968
Wheat	1.28	1.71	--	--	Wang et al., 1968
Wheat-soybean (1:1)	2.49	2.79	--	--	Wang et al., 1968
Idli (Black gram-rice)	1.84	1.81			Ananthachar and Desikachar, 1962
Idli	2.28	2.55	79.0	82.5	Khandwala et al., 1962
Idli	1.50	2.0	--	--	Rajalakshmi and Vanaja, 1967
Idli	1.99	1.84	--	--	van Veen et al., 1967
Ontjom (peanuts)	2.17	2.17	82.5	83.0	van Veen et al., 1968a
Ecuadorian rice	1.90	1.63	82.6	78.9	van Veen et al., 1968b

[a]Protein efficiency ratio.

Table III. Effect of *Rhizopus* fermentation on essential amino acids released by pepsin and pancreatin digestion of wheat.

Amino acid	Amino acid/ total essential amino acid: Unfermented	Fermented
	(mg/g)	
Lysine	119	137
Histidine	22	29
Threonine	173	156
Valine	72	64
Cystine + methionine	80	83
Isoleucine	69	72
Leucine	229	231
Tyrosine + phenylalanine	237	228

the animals. Since lysine is considered a limiting amino acid of wheat protein, the increase in available lysine would be nutritionally significant and can, in part, account for the increased PER value of wheat by fermentation. Improved PER values of horsebeans and soybeans were also noted by Kao and Robinson (1978). But they found that the rats fed fermented products ate more and gained more weight, which resulted in a higher PER value.

The protein quality of natto, a traditional Japanese food made by fermenting cooked whole soybeans with *Bacillus natto* Sawamura, has been investigated by a number of scientists in Japan. Conflicting results indicated that the nutritional quality of natto was greatly affected by processing and storage conditions. Ohta et al. (1964), however, found that natto fermentation had no effect on the protein quality of soybeans.

Although cereal and legume products made by natural bacteria and yeast fermentations are widely consumed around the world, the nutritional quality of these products, other than idli and natto, has not been investigated. Studies on natural fermentation to improve the protein quality of cereals and legumes have just begun in this country. Significant increases in relative nutritive value determined by *Tetrahymena pyriformis* W and in the amount of available lysine and methionine were noted in wheat, barley, rice, millet, maize, cowpeas, and chickpeas after a natural lactic acid fermentation (Hamad and Fields, 1979; Zamora and Fields, 1979; Tongnual et al., 1981). Increased nutritional value of corn meal fermented by yeasts was reported by Wang and Fields (1978). The relative nutritive value of corn meal as determined by the *T. pyriformis* W method was improved from 66.8 to 74.1% by fermenting with *Saccharomyces cerevisiae* Hansen at 25 °C for 3 days but from 66.8 to 82.6 by fermenting with *Candida tropicalis* (Castellani) Berkhout.

Complementary Effect of Mixed Proteins

The complementary effect of amino acids from one protein on the nutritive value of another is well recognized; therefore, an economical and practical way to improve protein quality as well as quantity is the combined use of complementary proteins. Among vegetable crops, legumes contained the highest amount of proteins. Although the legume protein is deficient in sulfur-containing amino acids, it is rich in lysine. Cereal grains, on the other hand, have an adequate amount of sulfur-containing amino acids, but their relatively low protein content is deficient in lysine. It seems logical that the proteins of legumes should complement the protein of cereal grains, as is often practiced in the making of traditional fermented foods. So even though the fermentation process may not have a significant effect on the protein quality, the process has made it easier and more convenient to blend legumes and grains for high protein and balanced amino acid foods. Ogi, a popular Nigerian food especially important in infant feeding and made by fermenting corn, was found to have a biological value so low that it did not support the growth of rats. When it was supplemented with 30% heated full-fat soy flour, however, its PER value increased threefold to almost as good as casein (Akinrele, 1966).

Vitamins

Microorganisms differ in their ability to synthesize vitamins. Some microorganisms are unable to synthesize any of the vitamins needed, and others may be able to synthesize some or all their vitamins. The vitamin content of various fermented foods, therefore, may be greater than that of the unfermented substrates. As shown in Table IV, niacin and riboflavin contents are greatly increased after tempeh fermentation.

Table IV. Vitamin content of legume and cereal foods before and after fermentation.

Food	Niacin B[a]	Niacin A[b]	Riboflavin B	Riboflavin A	Thiamin B	Thiamin A	Reference
	(μg/g)						
Tempeh:							
Soybean	17.5	65	2.6	8.6	7.8	5.8	Roelofsen and Talens, 1964
Soybean	9.0	44	0.6	4.9	2.2	1.3	Murata et al., 1967
Soybean	9.0	60	3.0	7.0	10.0	4.0	van Veen and Steinkraus, 1970
Wheat	46.0	135	0.4	3.2	3.2	3.0	Wang and Hesseltine, 1966
Idli	0	0	2.5	5.4	2.1	5.8	Rajalakshmi and Vanaja, 1967
Idli	0	0	1.4	0.8	0	0	van Veen et al., 1967
Ecuadorian rice	0	0	0.15	0.32	0	0	van Veen et al., 1968b

[a]Before fermentation.
[b]After fermentation.

Robinson and Kao (1978) also found that water soluble vitamins, except thiamin of horsebeans, chickpeas, and soybeans, were greatly increased after *Rhizopus* fermentation, which shows that *R. oligosporus* (tempeh mold) has a great synthetic capacity for both niacin and riboflavin. The mold might be able to synthesize thiamin, but the rate of synthesis is so slow that the organism utilizes readily available thiamin for maximum growth or other functions.

Lactic acid bacteria require vitamin-rich media for growth and would generally not be expected to increase vitamin levels in the food fermented with these organisms; nevertheless, slight increases in niacin, riboflavin, or thiamin have been reported in idli (Table IV). Zamora and Fields (1979) reported an 18% increase of riboflavin during a natural lactic acid fermentation of cowpeas, but thiamin decreased.

The most interesting and important finding, perhaps, was vitamin B12 in tempeh, ontjom, and kimchi because foods derived from plant materials are deficient in this essential nutrient. Darken (1953) found low levels of vitamin B12 in a few plants but traced the vitamin to contaminating microorganisms. Vitamin B12 is known to be synthesized only by microorganisms; however, molds have not been reported to produce vitamin B12.

Liem et al. (1977) found a fairly high amount of vitamin B12 in commercial tempeh from Canada and indicated that the major source of the vitamin was the result of contaminated bacteria which the authors isolated and identified as *Klebsiella*. They reported that tempeh made from pure mold isolated from the commercial tempeh contained nutritionally insignificant amounts of vitamin B12, confirming that the tempeh mold does not produce the vitamin. On the other hand, tempeh made with the mold and the bacterium, *Klebsiella*, isolated from commercial tempeh, had 150 ng of vitamin B12 per gram of tempeh. The presence of the mold does not interfere with the production of vitamin B12 by the bacteria, but presence of the bacteria requires longer fermentation time. Liem and his coworkers also demonstrated that soaking soybeans either with or without an acid bacterial fermentation did not increase the vitamin B12 content. The results indicated that tempeh made with pure mold fermentation under the hygienic practice adopted for food processing in this country has no nutritionally significant amount of vitamin B12, but there is a great potential to make vitamin B12-enriched tempeh with an inoculum containing *R. oligosporus* and a vitamin B12-producing bacterium.

The production of vitamin B12 in kimchi, Korean fermented vegetables, has been reported by Lee et al. (1960). The microorganisms responsible for producing the vitamin were identified as strains of

Pseudomonas (Kim et al., 1960) and *B. megatarium* de Bary (Kim and Chung, 1962). Recently, Ro et al. (1979) inoculated *Propionibacterium freudenreichii* van Niel ss. *shermanii* to natural kimchi fermentation and found the vitamin B12 content of kimchi was 47 ng/100 g without bacterial inoculation and 102 ng/100 g with bacterial inoculation after 1 week of fermentation. The level of vitamin B12, however, was reduced markedly by 2 and 3 weak of fermentation.

Thus, it is apparent that the native food may be quite variable in composition and also quite different from the laboratory product of pure culture fermentation.

Antiobiotics

An accidental and interresting discovery in studying the *R. oligosporus* protease was that the mold synthesizes one or more antibiotics of a glycopeptide nature (Wang et al., 1969). Subsequently, other species of *Rhizopus* and *A. elegans* (Eidam) Benjamin and Hesseltine used in the Orient for fermentation were also found to form the antibiotics (Wang et al., 1972). Antimicrobial tests (Wang et al., 1969) indicated that although the compound does not exhibit broad spectrum activity, it is very active against some gram-positive bacteria, including intestinal clostridia. This finding supports the view expressed by natives and also by some scientists that people who eat tempeh daily have fewer intestinal infections. More recently, Wong and Bau (1977) found that *M. purpureus* Went, the mold used for red rice fermentation, also produces an antibiotic which is active against many gram-positive bacteria. The antibiotics produced by these molds might benefit people whose diets are often nutritionally inadequate.

Conclusion

Fermentation technology has been used for centuries to upgrade plant materials and to yield a more acceptable food. The amino acid composition and the protein quality are generally not improved by fermentation, but in some cases the bioavailability of some essential amino acids is increased by fermentation. Many of the fermented foods made from mixtures of legumes and cereals, however, have a great nutritional advantage. Increases in vitamins are important, especially where vitamin deficiencies occur and where fortifying food with synthetic vitamins is not practiced. If microorganisms are properly screened and selected, the two most limited nutrients in plant materials, vitamin B12 and sulfur-containing amino acids, can be enriched by fermentation. Thus, the fermentation process has great potential to increase substantially the nutritional value of the world's most abundant food resources.

Not to be forgotten is the inherent value of these fermented foods beyond those of the major nutrients that have just been discussed. The most outstanding inherent value of these foods, perhaps, is their acceptability by the people, both socially and economically, the fermentation process has converted the unpalatable plant materials into interesting and attractive foods to add variety and flavor to otherwise monotonous staple dishes. Human needs can be satisfied by consuming a sufficient quantity of attractive foods with average nutritional value; however, a nutritionally perfect food is worthless if it is not accepted by the consumer.

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